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EFFECTS OF SALT STRESS ON THE GROWTH AND NITROGEN ASSIMILATION OF AFRICAN YAM BEAN (*SPHENOSTYLIS STENOCARPA)* (L)

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ABSTRACT: *The effect of salt stress on the growth and nitrogen assimilation of Sphenostylis stenocarpa (African yam bean) was investigated. There were five (5) treatments (0.01mol/l, 0.03mol/l, 0.07mol/l, 0.10mol/l) and distilled water without salt, served as control. Treatment started three (3) weeks after germination and was carried out once. The measurement of the plant started immediately after treatment was done for one month. The research lasted for three months. Four seeds were planted per plastic bucket and were thinned down to two. Salt stress significantly ($P \leq 0.05$) affected the growth and nitrogen assimilation of Sphenostylis stenocarpa (African yam bean), at higher salt (NaCl) concentration (0.07mol/l and 0.10mol/l), the length of shoot, number of branches, number of leaves, root biomass, shoot biomass, germination and the nitrogen content of the plant's leaf and soil were significantly ($p<0.05$) reduced. However, at a lower concentration (0.01mol/l to 0.03mol/l) the effect of salt stress on these parameters was not significant. The reduction in growth and nitrogen assimilation of Sphenostylis stenocarpa as a result of salt stress might be due to the deleterious effect of salt stress on the cellular as well as all other aspects of plant metabolism. Germination test was also carried out and lasted for only four days. At 0.07mol/l it tends to be retarded compared to the other treatment. The results obtained were discussed in the light of current literatures.*

KEYWORDS: Salt Stress, African Yam Bean, Sphenostylis Stenocarpa, Plant Metabolism, Nitrogen Assimilation

INTRODUCTION

Plants are exposed to a variety of abiotic stresses in nature and exhibit unique and complex responses to stresses depending on their degree of plasticity involving many morphological, cellular, anatomical and physiological changes (Fahad *et al*., 2015). Although plant response to salinity depends on several factors; nevertheless, phytohormones are thought to be the most important endogenous substances that are critical in modulating physiological responses that eventually lead to adaptation to salinity.

Responses usually involves fluctuations in the levels of several phytohormones, which relates with changes in expression of genes involved in their biosynthesis and the responses they regulate (Achard *et al*., 2006). In most of the cases, the negative effects of salinity have been attributed to increase in Na^+ and Cl^-ions in different plants hence these ions produce the critical conditions for plant survival by intercepting different plant mechanisms (Munns, 2002a). Although both Na^+ and $Cl^-$are the major ions which produce many physiological disorders in plants, $Cl^-$is the most dangerous (Tavakkol *et al*., 2010).

Soil salinity is one of the major constraints responsible for low agriculture in Pakistan (Zou *et al*., 1995). Selection of salt tolerant cultivar is one of the most effective methods to increase

the productivity of such soils (Pitman and Lauchi, 2002). The major inhibitory effects of salinity on plant growth and yield has been attributed to: (1) osmotic effect (2) ion toxicity (3) nutritional imbalance leading to reduction in photosynthetic efficiency and other physiological disorders (Munns, 2002). Adverse effects of salinity on seed germination and seedling growth as well as some physiological activities of cultivated plant species have been extensively investigated in Pakistan (Xu *et al*., 2011). Salinity is one of the most brutal environmental factors limiting the productivity of crop plant because most of the crop plants are sensitive to results caused by high concentration of salts in the soil (Ashraf, 1994). A considerable amount of land in the world is affected by salinity which is increasing day by day (Pitman and Lauchi, 2002; Munns and Tester, 2008). On the other hand, increased salinity of agricultural land is expected to lead up to 50% loss of cultivable lands by the middle of the twenty-first century (Parida and Das, 2005).

Plant growth and seed germination is an important and vulnerable stage in the life cycle of any plant and determines seedling establishment and plant growth. Despite the importance of seed germination under salt stress, the mechanism of salt tolerance in seeds is relatively poorly understood, especially when compared with the amount of information currently available about salt tolerance physiology and biochemistry in plants (Khodarampour *et al*., 2012). Salt stress causes reduced cell turgor and depressed rates of roots and leaf elongation, suggesting that environment salinity acts primarily on water uptake (Ashraf, 1994). Furthermore, high intracellular concentrations of both Na^+ and Cl^- can inhibit the metabolism of dividing cells, retarding germination and even leading to seed death (Parida and Das, 2005).

High Salinity causes both hypertonic and hyperosmotic stress and can lead to death of plants (Hasegawa *et al*., 2000). It is reported that plants growing under saline condition are affected in three ways: reduced water potential in root zone causing water deficit, phytotoxicity of ions such as Na^+ and Cl^- and nutrient imbalance depressing uptake and transport of nutrients. Na^+ competes with K^+ for binding sites essential for cellular functions (Munns, 2002a). Excess salt concentration also enhances the osmotic potential of soil matrix which restricts the water uptake by plants. Sodium is the primarily toxic ion, because it interferes with K^+ uptake as well as disturbs stomatal regulation which ultimately causes water loss and necrosis. On the other hand, Cl^- induces chlorotic toxicity symptoms due to impaired production of chlorophyll. In plant cells, Cl^- is required for the regulation of some enzyme's activities in the cytoplasm. It is also a co-factor photosynthesis and is involved in turgor and pH regulation.

The aim of this study is to evaluate the effects of different levels of salt (NaCl) concentrations on the growth and nitrogen assimilation of African yam bean (*Sphenostylis Stenocarpa*).

MATERIALS AND METHODS

Seed Collection

The African yam bean used for this work was bought from the New Market, Aba, Abia State.

Potting Medium

The soil samples used for this work were collected in front of Screen House of the College of Crop and Soil Sciences, Michael Okpara University of Agriculture, Umudike. Twenty-five

buckets were used, which were perforated uniformly, labelled and arranged according to the treatments used. Each bucket was filled two-third with the soil sample (Loamy soil) and then watered properly.

Treatment Application

The salt treatment was done using Sodium Chloride (NaCl). The treatments used: 0.01mol/l, 0.03mol/l, 0.07mol/l and 0.10mol/l which is the highest concentration and distilled water without salt was given as control. The treatment was given three (3) weeks after planting using the method of Motahari *et al*., 2005. The treatment was done only once.

Growth Measurement Parameters

The measurement of African yam bean started immediately after treatment and was taken once in a week for one month. The heights of the plants were measured using meter rule and the number of leaves and branches was done manually.

Plant Height (cm)

The heights of the plants were measured using the meter-rule and recorded.

Number of Leaves

The number of leaves were counted manually and recorded.

Number of Branches

The number of branches were also counted manually and then recorded. After one month, the shoot of the plant was separated from the root. The roots were washed, dried and weighed.

Determination of Nitrogen Content

The nitrogen content was determined by Kjeldahl method described by James (1995). One-half gram (0.5g) of each sample was mixed with 10mls of concentrated sulphuric acid AR grade (Analytical Reagent Grade) in a Kjeldahl flask. A tablet of selenium catalyst was added to it and the mixture was digested (heated) under a fume cupboard until a clear solution was obtained in a separate flask. The acid and other reagents were digested but without sample, to form the black control.

All the digests were carefully transferred to a 100mls portion of each digest and were mixed with equal volume of 45% NaOH solution in Kjeldahl distilling unit. The mixture was distilled and the distillate collected into 10mls of 4% Boric acid solution containing three (3) drops of mixed indicators (bromocresol green, methyl red). A total of 50mls distillate was obtained and titrated against 0.02m H_2SO_4 solution. Titration was done from the initial green colour to deep red end point.

The nitrogen concentration calculation is shown below:

$$\%N_2 = \frac{(100 \times N \times 14\ Vf)\ T}{W \times 1000 \times Va}$$

Where:

W = Weight of sample analyzed

N = Concentration of H_2SO_4

Vf = Total volume of digest

Va = Volume of digest distilled

Seed Germination (%)

Twenty-five petri-dishes and filter papers were used to carry out the germination test of *Sphenostylis stenocarpa* which was arranged based on their treatments. The treatments used were 0.01mol/l, 0.03mol/l, 0.07mol/l, 0.10mol/l and the distilled water without salt served as the control. Four seeds were put inside each petri-dish and the treatment was applied on it every day till it germinated. The rate of germination was calculated using the method by Djavnshir and Pourbeik (1976).

$$\text{Germination Rate} = \frac{\text{Total number of seed germinated}}{\text{Number of days in the germinator}}$$

Percentage germination was calculated with the formula:

$$\text{Germination} = \frac{\text{Total number of seeds germinated}}{\text{Total number of seeds planted}} \times \frac{100}{1}$$

RESULTS

The results of the salt stress on the growth and nitrogen assimilation of *Sphenostylis stenocarpa* were summarized in tables 1-4.

Effects of Salt Stress on Length of Shoot, Number of Leaves and Number of Branches of African Yam Bean

There was significant decrease ($P \leq 0.05$) in height, number of leaves and number of branches of African yam bean with respect to the salt (NaCl) treatment. The effect increased with increasing salt (NaCl) treatment.

Table 1: The Effect of Salt Stress on Length of Shoot, Number of Leaves and Number of Branches of African Yam Bean (*Sphenostylis Stenocarpa)*

Concentration (MOL/L)	Length of shoot (CM)	Number of leaves	Number of branches
Control	194.50 ± 13.63^{ab}	45.25 ± 2.36^{a}	16.00 ± 0.469^{a}
0.01	334.53 ± 46.39^{c}	82.50 ± 20.40^{b}	29.00 ± 10.68^{b}
0.03	415.73 ± 59.18^{c}	101.75 ± 23.37^{b}	34.50 ± 9.67^{b}
0.07	239.70 ± 16.73^{b}	48.50 ± 12.12^{ab}	17.75 ± 6.65^{ab}
0.10	203.75 ± 25.04^{bc}	52.25 ± 16.91^{b}	17.00 ± 9.05^{b}

Effect of Salt Stress on Root and Shoot Biomass of African Yam Bean (*Sphenostylis Stenocarpa*)

Salt stress had no significant effect on the fresh weight of the shoot and root biomass of African yam bean at lower concentration (0.01mol/l, 0.03mol/l and 0.07mol/l), but at higher concentration of 0.10mol/l, there was a significant effect. However, salt stress significantly ($P \leq 0.05$) reduced the dry weight (biomass) of the shoot and root of African yam bean (Table 2). Generally, increasing salt concentration caused increased reduction in the biomass of the shoot root of African yam bean.

Table 2: Effect of Salt (NaCl) Treatment on Shoot and Root Biomass

Concentration (MOL/L)	Shoot biomass (g) Fresh weight	Dry weight	Root biomass (g) Fresh weight	Dry weight
Control	1.281 ± 0.12^{a}	1.175 ± 0.15^{a}	0.373 ± 0.13^{a}	0.313 ± 0.12^{a}
0.01	1.075 ± 0.11^{ab}	0.956 ± 0.08^{ab}	0.231 ± 0.10^{ab}	0.259 ± 0.16^{b}
0.03	1.075 ± 0.03^{ab}	0.690 ± 0.16^{b}	0.338 ± 0.27^{ab}	0.254 ± 0.13^{b}
0.07	0.74 ± 0.11^{ab}	0.579 ± 0.16^{b}	0.188 ± 0.06^{ab}	0.135 ± 0.06^{c}
0.10	0.693 ± 0.26^{b}	0.449 ± 0.16^{b}	0.226 ± 0.07^{ab}	0.241 ± 0.14^{bc}

Effect of Salt Stress (NaCl) on Nitrogen Assimilation

Salt stress significantly ($P \leq 0.05$) affected the nitrogen assimilation of African yam bean. The nitrogen content of the soil was reduced due to the treatment (Table 3).

Generally, there was significant decrease in soil nitrogen content as the concentration of treatment increased.

Effect of Salt Stress on Germination of African Yam Bean

Salt stress significantly at ($P \leq 0.05$) affected the germination of the seeds of African yam bean at higher concentrations (0.07mol/l and 0.10mol/l). The highest germination rate was in the control, while 0.07 mol/l had the least germination rate.

Table 4: Effect of Salt Stress on Percentage Germination of African Yam Bean

Concentration (MOL/L)	Germination
Control	70 ± 2.59^{a}
0.01	65 ± 2.50^{a}
0.03	50 ± 2.56^{a}
0.07	45 ± 2.02^{b}
0.10	30 ± 1.7^{ab}

DISCUSSION

Effects of Salt Stress on Growth of African Yam Bean

The results showed that high concentration of Sodium Chloride (NaCl) significantly ($P< 0.05$) reduced the growth of African yam bean seedlings. Increased concentration of salt had an increased reduction in the growth of African yam bean. The investigations are in agreement with similar studies in different plants (Ikhajiagbe *et al*., 2007a; Maggio *et al*., 2007; Ikhajiagbe *et al*., 2007b; Parida *et al*., 2005 and Motahari *et al*., 2005).

According to Munns (2002b), the mechanisms by which salinity affects growth of plant depend on the time scale over which the plant is exposed to salt. The number of branches, number of leaves, length of the plant, shoot and root biomass were significantly ($P< 0.05$) reduced. The results gotten from this work showed that African yam bean is less sensitive to salt stress at lower salt (NaCl) concentration. African yam bean was able to adjust its physiological activities to salt stress condition (Ulfat *et al*., 2007; Parida and Das, 2005) which would result in improved plant growth. The result also showed that African yam bean was tolerant to various salt concentrations. This is in agreement with work done by Lauchi *et al*. (2007) and Rumbaugh *et al*. (1993).

Effects on Nitrogen Assimilation

Higher salt (NaCl) concentrations significantly reduced ($P< 0.05$) the amount of nitrogen fixed into the soil and taken up by the root of African yam bean from the soil. This shows the sensitivity of nitrogenase activity to salt stress. The root is the first organ to experience salt stress, as the root system is involved in water absorption and minerals uptake from the soil. Soil salinity caused reduction in the rate of nitrogen absorption in the form of nitrates. During salt stress in plants, nitrate reductase (NR) activity decrease in many plants. In chickpeas, salinity inhibited nitrogen fixation by reducing nodulation and nitrogenase activity (Soussi *et al*., 1998).

Effects on Germination

Salt stress significantly reduced ($P< 0.05$) the germination of African yam bean. The least rate of germination occurred at 0.07 mol/l as compared to the other treatments.

The observations in this present study are in agreement with the works of other researchers on the effects of salt stress on seed germination of various crops like *Oryza sativa* (XU *et al*., 2011), *Triticum aestivum* (Akbarimaghaddam *et al*., 2011), *Zea mays* (Carpi *et al*., 2009; Khodarahmpour *et al*., 2012), *Brassica spp* (Ibra *et al*., 2003; Ulfat *et al*., 2007) and *Glycine max* (Essa, 2002).

CONCLUSION

This study observed a decrease in the length of the plant which caused poor growth of the plant. The study observed that the sodium chloride (NaCl) treatment 0.03mol/l and 0.01mol/l had no effect on African yam bean, however, at concentration of 0.07mol/l and 0.10mol/l, the growth of African yam bean was affected.

RECOMMENDATION

In areas with low salinity, African yam bean may be cultivated whereas at higher concentration, African yam bean should not be cultivated, this is due to the fact that African yam bean cannot do well in such environment.

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